

APPENDIX A:
PENDING CLAIMS AS OF PRELIMINARY AMENDMENT

18. (Amended) A method of screening for a modulator of calpain function comprising:

- a) obtaining a calpain polypeptide;
- b) determining a standard activity profile of the calpain polypeptide;
- c) contacting the calpain polypeptide with a putative modulator; and
- d) assaying for a change in the standard activity profile.

19. The method of claim 18, wherein the calpain polypeptide is a calpain 10 polypeptide.

20. The method of claim 18, wherein obtaining the calpain polypeptide comprises expressing the polypeptide in a host cell.

21. The method of claim 20, wherein the calpain polypeptide is isolated away from the host cell prior to contacting the calpain polypeptide with the putative modulator.

50. The method of claim 19, wherein the standard activity profile of the calpain 10 polypeptide is determined by measuring the binding of the calpain 10 polypeptide to a synthetic substrate.

51. The method of claim 50, wherein the synthetic substrate is Suc-Leu-Tyr-AMC.

52. A method of screening for a modulator of calpain function comprising:

- a) obtaining an calpain polypeptide;
- b) contacting the calpain polypeptide with a putative modulator; and
- c) assaying for modulation of calpain function by the putative modulator.

53. The method of claim 52, wherein the calpain polypeptide is a calpain 10 polypeptide.

54. The method of claim 53, wherein the calpain 10 polypeptide has a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

55. The method of claim 52, further comprising determining a standard activity profile of the calpain polypeptide.

56. The method of claim 55, wherein the standard activity profile of the calpain 10 polypeptide is determined by measuring the binding of the calpain 10 polypeptide to a synthetic substrate.

57. The method of claim 56, wherein the synthetic substrate is Suc-Leu-Tyr-AMC.

58. The method of claim 56, wherein assaying for modulation of calpain function comprises assaying for a change in the standard activity profile.

59. The method of claim 52, wherein obtaining the calpain polypeptide comprises expressing the polypeptide in a host cell.

60. The method of claim 59, wherein the calpain polypeptide is isolated away from the host cell prior to contacting the calpain polypeptide with the putative modulator.

61. The method of claim 52, wherein obtaining the calpain polypeptide comprises obtaining a cell containing the polypeptide.

62. The method of claim 61, wherein the cell is a pancreatic cell, a muscle cell, an adipose cell, or a liver cell.

63. The method of claim 62, wherein the cell is a pancreatic cell.

64. The method of claim 63, wherein the pancreatic cell is comprised in an isolated pancreatic islet.

65. The method of claim 63, wherein the cell is a β -cell.

66. A method of screening for a modulator of calpain function comprising:

- obtaining an calpain-encoding nucleic acid segment;
- determining a standard transcription and translation activity of the calpain nucleic acid sequence;
- contacting the calpain-encoding nucleic acid segment with a putative modulator;
- maintaining the nucleic acid segment and putative modulator under conditions that normally allow for calpain transcription and translation; and
- assaying for a change in the transcription and translation activity.

67. The method of claim 66, wherein the calpain-encoding nucleic acid segment encodes calpain 10.

68. A calpain modulator prepared by a process comprising screening for a modulator of calpain function comprising:

- obtaining an calpain polypeptide;
- determining a standard activity profile of the calpain polypeptide;
- contacting the calpain polypeptide with a putative modulator; and
- assaying for a change in the standard activity profile.

69. The modulator of claim 68, wherein obtaining the calpain polypeptide comprises expressing the polypeptide in a host cell.

70. The modulator of claim 68, wherein the calpain polypeptide is a calpain 10 polypeptide.

71. The modulator of claim 69, wherein the calpain polypeptide is isolated away from the host cell prior to contacting the calpain polypeptide with the putative modulator.

72. The modulator of claim 68, wherein the modulator of calpain function is a modulator of a calpain polypeptide.

73. The modulator of claim 72, wherein the calpain polypeptide is a calpain 10 polypeptide.

74. The modulator of claim 73, wherein the calpain 10 polypeptide has a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

75. The modulator of claim 68, wherein the modulator of calpain function is an agonist or antagonist of a calpain polypeptide.

76. The modulator of claim 75, wherein the modulator of calpain function is an inhibitor of a calpain polypeptide.

77. The modulator of claim 76, wherein the modulator inhibits calpain I and/or calpain II.

78. The modulator of claim 76, wherein the modulator is calpeptin.

79. The modulator of claim 76, wherein the modulator is calpain inhibitor 2 (ALLM).

80. The modulator of claim 76, wherein the modulator of calpain function is a protease inhibitor.

81. The modulator of claim 80, wherein the protease inhibitor is a thiol protease inhibitor.

82. The modulator of claim 81, wherein the thiol protease inhibitor is E-64-d.

83. The modulator of claim 68, further defined as a method comprising inhibiting calpain activity in a β -cell with a modulator of calpain function.

84. The modulator of claim 68, further defined as a method comprising stimulating calpain activity in a muscle cell or fat cell with a modulator of calpain function.

85. The modulator of claim 68, further defined as a method comprising stimulating calpain activity in a fat cell or muscle cell with a modulator of calpain function and inhibiting calpain activity in a β -cell with a modulator of calpain function.

86. A calpain modulator prepared by a process comprising screening for a modulator of calpain function comprising:

- a) obtaining a calpain-encoding nucleic acid segment;
- b) determining a standard transcription and translation activity of the calpain nucleic acid sequence;
- c) contacting the calpain-encoding nucleic acid segment with a putative modulator;
- d) maintaining the nucleic acid segment and putative modulator under conditions that normally allow for calpain transcription and translation; and
- e) assaying for a change in the transcription and translation activity.

87. The method of claim 86, wherein the calpain-encoding nucleic acid segment encodes calpain 10.

88. A method of treating diabetes by modulating the function of one or more calpains in at least one of a β -cell, muscle cell, or fat cell with a modulator of calpain function, wherein the modulator is prepared by a process comprising screening for a modulator of calpain function comprising:

- a) obtaining a calpain-encoding nucleic acid segment;
- b) determining a standard transcription and translation activity of the calpain nucleic acid sequence;
- c) contacting the calpain-encoding nucleic acid segment with a putative modulator;
- d) maintaining the nucleic acid segment and putative modulator under conditions that normally allow for calpain transcription and translation; and

e) assaying for a change in the transcription and translation activity.

89. The method of claim 88, wherein the calpain-encoding nucleic acid segment encodes calpain 10.

90. A method of treating diabetes by modulating the function of one or more calpains in at least one of a β -cell, muscle cell, or fat cell with a modulator of calpain function, wherein the modulator is prepared by a process comprising screening for modulators of calpain function comprising:

- a) obtaining an calpain polypeptide;
- b) determining a standard activity profile of the calpain polypeptide;
- c) contacting the calpain polypeptide with a putative modulator; and
- d) assaying for a change in the standard activity profile.

91. The method of claim 90, wherein the modulator of calpain function is a modulator of a calpain polypeptide.

92. The method of claim 91, wherein the calpain polypeptide is a calpain 10 polypeptide.

93. The method of claim 92, wherein the calpain 10 polypeptide has a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, or SEQ ID NO:18.

94. The method of claim 91, wherein the modulator of calpain function is an agonist or antagonist of a calpain polypeptide.

95. The method of claim 91, wherein the modulator of calpain function is an inhibitor of a calpain polypeptide.

96. The method of claim 95, wherein the modulator inhibits calpain I and/or calpain II.

97. The method of claim 95, wherein the modulator is calpeptin.

98. The method of claim 95, wherein the modulator is calpain inhibitor 2 (ALLM).

99. The method of claim 95, wherein the modulator of calpain function is a protease inhibitor.

100. The method of claim 99, wherein the protease inhibitor is a thiol protease inhibitor.

101. The method of claim 100, wherein the thiol protease inhibitor is E-64-d.

102. The method of claim 90, further defined as a method comprising inhibiting calpain activity in a β -cell with a modulator of calpain function.

103. The method of claim 90, further defined as a method comprising stimulating calpain activity in a muscle cell or fat cell with a modulator of calpain function.

104. The method of claim 90, further defined as a method comprising stimulating calpain activity in a fat cell or muscle cell with a modulator of calpain function and inhibiting calpain activity in a β -cell with a modulator of calpain function.

105. The method of claim 90, wherein the standard activity profile of the calpain 10 polypeptide is determined by measuring the binding of the calpain 10 polypeptide to a synthetic substrate.

106. The method of claim 105, wherein the synthetic substrate is Suc-Leu-Tyr-AMC.

107. The method of claim 105, wherein assaying for modulation of calpain function comprises assaying for a change in the standard activity profile.

108. The method of claim 90, wherein obtaining the calpain polypeptide comprises expressing the polypeptide in a host cell.

109. The method of claim 108, wherein the calpain polypeptide is isolated away from the host cell prior to contacting the calpain polypeptide with the putative modulator.

110. The method of claim 90, wherein obtaining the calpain polypeptide comprises obtaining a cell containing the polypeptide.

111. The method of claim 110, wherein the cell is a pancreatic cell, a muscle cell, an adipose cell, or a liver cell.

112. The method of claim 111, wherein the cell is a pancreatic cell.

113. The method of claim 112, wherein the pancreatic cell is comprised in an isolated pancreatic islet.

114. The method of claim 112, wherein the cell is a β -cell.